

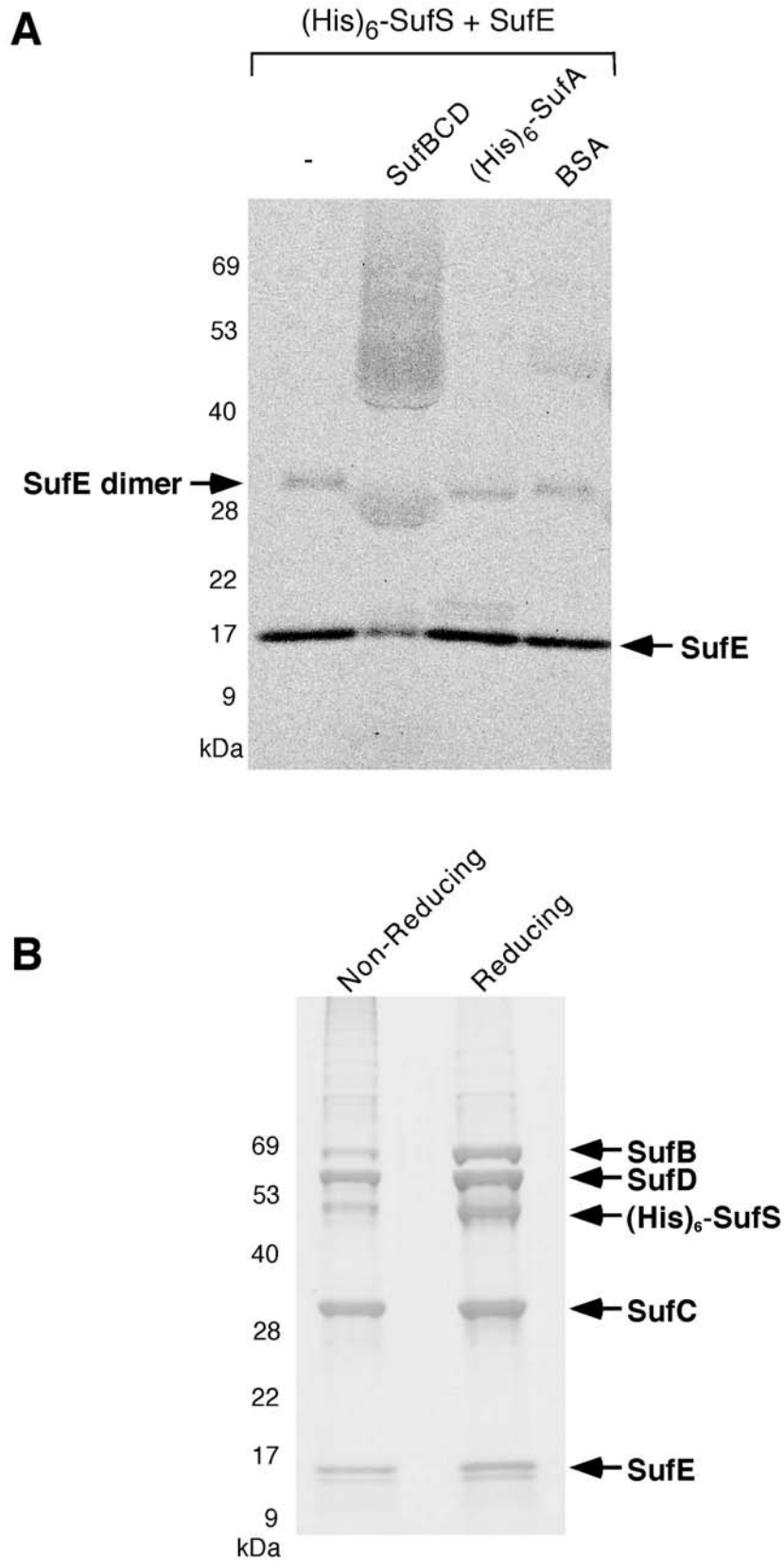
SUPP. FIG. 1. Formation of higher order SufS, SufE, and SufBCD complexes during sulfur transfer. *A*, (His)₆-SufS (1 μM) and SufE (20 μM) were mixed with 20 μM SufBCD, (His)₆-SufA, or BSA and incubated in the presence of ³⁵S-L-cysteine. Samples were separated on a non-reducing 10-20% Tris-glycine gel. *B*, Selective depletion of the monomeric forms of SufS, SufB, and SufE under non-reducing conditions. 10 μM (His)₆-SufS, 20 μM SufE, and 10 μM SufBCD were mixed and reactions were initiated by addition of 1 mM L-cysteine and 400 μM FeCl₃ in a 20 μl reaction volume. Reactions were trapped by the addition of 5 μl of 100% TCA and incubation for 20 min on ice. Precipitated protein was resuspended in 1M Tris, pH 8.0, 0.1% SDS, and 20 mM iodoacetamide, split into two equal samples, and separated on a 10-20% Tris-glycine gel under reducing (250 mM βME) or non-reducing conditions.

SUPP. FIG. 2. Covalently linked SufS-SufE dimer forms during sulfur transfer. (His)₆-SufS (1 μM) was mixed with SufE (20 μM), incubated in the presence of ³⁵S-L-cysteine, and trapped by TCA precipitation by the addition of 9 μl of 100% TCA and incubation for 20 min on ice. Precipitated protein was resuspended in 1M Tris, pH 8.0, 0.1% SDS, and 20 mM iodoacetamide. Samples were separated on a non-reducing 10-20% Tris-glycine gel.

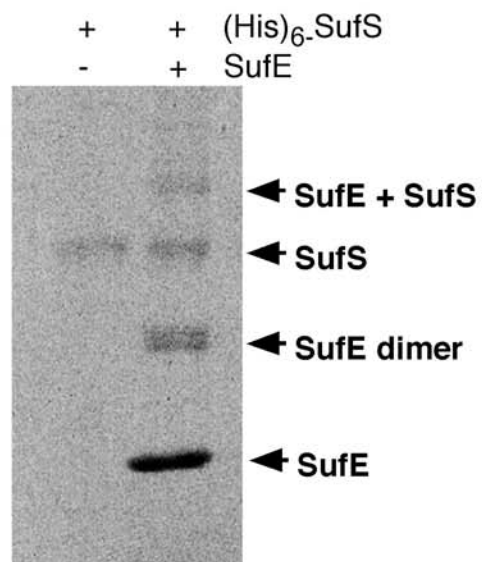
SUPP. FIG. 3. SufS to SufE sulfur transfer is resistant to reductant. (His)₆-SufS (1 μM) was mixed with SufE (6 μM), incubated in the presence of ³⁵S-L-cysteine and increasing amounts of DTT. The transfer reaction was terminated by separation on a G50

ProbeQuant spin column (Amersham-Pharmacia) as described in Experimental Procedures. Samples were separated on a non-reducing 10-20% Tris-glycine gel.

Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3

